

Syringodium isoetifolium Fosters an Antioxidant Defense System, Modulates Glycolytic Enzymes and Protects Membrane Integrity in DEN-induced Hepatocellular Carcinoma in Albino Wistar Rats

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ABSTRACT

Background: *Syringodium isoetifolium* seagrass has bioactive constituents with potential pharmacological uses, but their use is limited owing to scarce scientific evidence. The *in vivo* anti-cancer activity of *Syringodium isoetifolium* against DEN-induced hepatocellular carcinoma in Wistar albino rats is described in this work for the first time. **Materials and methods:** Wistar albino rats were used as test subjects to examine the anti-cancer properties of *Syringodium isoetifolium* against DEN-induced hepatocellular carcinoma at the dose of 50 mg/kg body weight. The experimental rats were split into five groups (Group I-V). Except for group I, remaining all animals received DEN and Phenobarbitone during the experiment. Group I and Group II acted as normal and diseased control groups respectively. The extracts were administered to the satellite group III and IV orally with the dose of 250 and 500 mg/kg body weight respectively. 5 fluorouracil 20mg/kg was administered to group V orally and considered as a standard. The total experimental period lasted for 14 weeks. **Results:** The findings show that *Syringodium isoetifolium* significantly reduces liver tumor volume, burden and numbers in experimental rats ($p < 0.05$) when compared to the control group. Besides, the extracts treated groups restored the pathological parameters close to normal values ($p < 0.05$). The histological analysis also showed that the extract-treated animals' livers had recovered their normal architecture. **Conclusion:** The study concludes that *Syringodium isoetifolium* inhibits the cancer growth in hepatocellular carcinoma by altering the antioxidant defense system, glycolysis and protecting the membrane architecture by inhibiting the elevated levels of haematological, biochemical parameters and biomarker values.

Keywords: *Syringodium isoetifolium*, Hydroalcoholic extract, Wistar albino rats, Toxicology report, Hepatocellular carcinoma.

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Received: 27-09-2022;

Revised: 20-03-2023;

Accepted: 15-06-2023.

INTRODUCTION

By 2030, 17 million individuals will die from cancer, with 26 million of those cases occurring largely in developing and impoverished nations.^{1,2} According to the International Agency for Cancer Research, 7,84,821 persons died from cancer in India alone in 2018, accounting for 6% of all cancer-related fatalities.³ One of the cancer forms with the greatest rate of growth in India is liver cancer, a potentially fatal condition.⁴ More than 1 million people worldwide suffer from this prevalent dangerous cancer, which resulted in 800,000 fatalities in 2016.⁵ There was

a 114.0% rise in liver cancer incident cases.⁶ Despite notable advancements in treatments and prevention measures, it has been a continuing fight around the world.⁷ The cancer treatment options include surgery, radiation, chemotherapy, targeted therapy, immunotherapy, bone marrow transplant and hormone therapy which are among the possible cancer treatments.^{8,9} One of the main problems with the development of traditional anti-cancer medications is the onset of multidrug resistance and relapse.¹⁰ Herbal medicines to treat disease and enhance the general health and well-being of a person.¹¹ In the recent past, evidence suggests that they should be employed as an alternative to traditional therapies.^{12,13} Cancer therapy is increasingly being acknowledged as an effective adjunct to the use of medicinal plants and their phytoconstituents.¹⁴ Seagrasses are blooming plants that flourish in bays and other shallow coastal environments.¹⁵ The biomass of seagrass has been utilized



DOI: 10.5530/ijper.57.3s.78

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frequently as food and drug by coastal indigenous people.¹⁶ In folk medicine, seagrasses have been employed for many therapeutic purposes such as skin diseases, fever, wounds, stomach problems, muscle pains and as a remedy against different kinds of rays.¹⁷ They also provide different pharmacological activities like antioxidant,¹⁸ anti-microbial,¹⁹ anti-viral,²⁰ stomach problems,²¹ anti-diabetic,²² wounds,²³ tranquilizer,²⁴ anti-cancer²⁵ activities etc. These plants have bioactive constituents with potential pharmacological uses, but their use is limited owing to scarce scientific evidence. *Syringodium isoetifolium* belonging to the family Cymodoceaceae also referred to as noodle seagrass, is a flowering plant that grows underwater in marine habitats. The hydroalcoholic extract of *Syringodium isoetifolium* showed *in vitro* anti-proliferative action on liver cancer cells, demonstrating that *Syringodium* species continuously affect the group of HepG2 human cancer cell line²⁶ and induce apoptosis.²⁷ The *in vivo* anti-cancer activity of *Syringodium isoetifolium* against DEN-induced hepatocellular carcinoma in Wistar albino rats is described in this work for the first time. The findings suggest that the hydroalcoholic extract of *Syringodium isoetifolium* augments the antioxidant defense system, alters hematological parameters and liver glycolytic enzymes in DEN-induced cancers. The research's findings mark a significant turning point in the natural medicine used as an anti-cancer medication development process (Figure 1).

MATERIALS AND METHODS

Materials

Seagrass extract preparation

The seagrass leaves were collected, dried in the shade, powdered and extracted with hydroalcoholic solvent (30:70) using cold maceration. The hydroalcoholic solvent extracted all the bioactive phytoconstituents from *Syringodium isoetifolium* seagrass. Here, 450 mL of cold solvents were added to the leaf powder, and they were gently shaken in a closed container for 24 hr which was later filtered and collected. The solvent was removed in a rotary evaporator at 60°C.^{28,29} The solid seagrass extract has been kept safe for further use. The formula below was used to determine the yield.

$$(W_1 \times 100) / (W_2 \times 100) = \text{Percentage Yield}$$

Here, W_1 is the weight of the extract after the solvent is removed, and W_2 is the sample's dry weight.

The seagrass was authenticated by Dr M.U. Sharief, Scientist E and Head, Botanical Survey of India, Southern Regional Centre, T.N.A.U Campus, Coimbatore – 641003. The Voucher specimen number of the *Syringodium isoetifolium* is BSI/SRC/5/23/2021/Tech/372.

Methods

Phytochemical analysis

The collected dried hydroalcoholic extract of *Syringodium isoetifolium* was qualitatively tested for the presence of different phytochemicals by chemical and HR-LCMS techniques. Chemical method was performed with different reagents using standard procedures to know the phytochemicals present in the leaf's extracts of *Syringodium isoetifolium*. Whereas, HR-LCMS chromatography can isolate the different secondary metabolites present in the plant extract and mass spectra identified the phytoconstituents through their mass value and their typical mass fragmentation patterns. This is a powerful tool for analyzing and detection of phytochemicals in plant products.

Chemicals employed

All of the compounds used in this experiment were of analytical quality, whereas the Diethyl Nitrosamine (DEN) was acquired from Sigma Aldrich, Bangalore.

Ethical approval

The entire method was carried out in accordance with the OECD's guidelines for animal experimental protocols, and the study was only carried out after receiving approval from the institutional animal ethics committee (Registration number: GSP/IAEC/2022/12/02).

Experimental animals

The study was employed on 2-month-old Wistar rats (Male) that ranged in weight from 100 to 150 g. The rats were kept in the animal house at a constant temperature of 22°C with a 12 hr light/12 hr dark cycle. Rats were given a commercial pellet meal and unlimited amounts of water during the research.³⁰ The blood and liver of the test animals were analyzed at the end of the investigation.

Acute toxicity

According to OECD rules 423, about 9 Wistar albino female rats were randomly divided into 3 groups, each of which had 3 rats. All of the animals received a graduated dosage of *Syringodium isoetifolium* hydroalcoholic extracts. Stepwise administration of a single fixed dose of *Syringodium isoetifolium* hydroalcoholic extracts (500, 1000 and 2000 mg/kg body weight) to each group of rats. The rats were starved for 4 hr, later the dosages were administered and observations were made twice daily for 14 days. Mortality, behavioral abnormalities, including irritability, restlessness, fear, variations in the eye, hair, skin color and alterations in food and fluid consumption were noted.^{31,32}

DEN preparation

Upon arrival, DEN is stored at -10°C to prevent its decomposition. The DEN solution was prepared by dissolving DEN (50 mg/kg body weight) in 0.9% NaCl solution and acidified with acetic acid (pH 5.0). The DEN solution was administered intraperitoneally using a 1 mL disposable syringe equipped with a 26-gauge 3/8-inch needle.³³

Phenobarbitone solution preparation

The phenobarbitone tablet was purchased in a retail pharmacy shop in Coimbatore, India. Phenobarbitone 60 mg, gum acacia was dissolved in 100 mL of distilled water. Here, gum acacia act as an excipient. Phenobarbitone was used to promote cancer in Groups II-V animals after DEN administration. The dose was given to the animals from day 1 to 98.³⁴

Effect of *Syringodium isoetifolium* on DEN induced hepatocellular carcinoma

The experimental animals were grouped as follows;

Group I acted as normal control and received saline administration throughout the testing period.

Group II- DEN+PB was used as a diseased control.

Group III-DEN+PB and HAESI extract at 250 mg/Kg b.wt. was administered every day for 14 weeks (low dose).

Group IV- DEN+PB and HAESI extract was administered every day for 14 weeks at a dose of 500 mg/Kg b.wt. (high dose).

Group V- DEN+PB and 5-Fluorouracil (20 mg/Kg b.wt.) was administered intraperitoneally twice a week for 28 days (post-induction).

Animals in Group II-V received a single intraperitoneal dosage of DEN (50 mg/kg body weight). The body weight, liver weight, tumor volume, burden and numbers etc., were evaluated in the experimental animals till 14 weeks. Later, blood samples were collected from the experimental animals through the retro-orbital plexus, the liver was removed and preserved in 10% formaldehyde solution to analyses the biochemical and histopathological features.³⁵

Histopathology study

At the culmination of the study, all animals were sacrificed, and the livers were removed, cleaned to eliminate fat, and weighed. Hematoxylin-eosin was used to stain the prepared paraffin-embedded blocks used to generate the liver slices. These slices were examined using light microscopy.³⁶

Biochemical and hematological parameters

The technique of Verma *et al.*³⁷ was used to evaluate hematological parameters such as Red Blood Cells (RBC), White Blood Cells (WBC), and Hemoglobin (Hb). The approach described

by Sannad *et al.*³⁸ and Wang *et al.*³⁹ was used to estimate the biochemical parameters such as total protein and total bilirubin. Chang *et al.*⁴⁰ and Zhang *et al.*⁴¹ techniques were used to assess the SGOT, SGPT and serum ALP. The technique of Musso *et al.*⁴² was used to determine the levels of urea, uric acid, and creatinine in serum. According to the methods of Soska *et al.*⁴³ and Shahriari *et al.*,⁴⁴ the total cholesterol, triglyceride, HDL cholesterol, SOD, Catalase, GPX, and LPO were evaluated. According to Iynedjian *et al.*⁴⁵ and Cullen KS *et al.*,⁴⁶ the liver's glycolytic enzymes-hexokinase, glucokinase, glucose 6 phosphatase and fructose 1,6 diphosphatase-were evaluated. Alpha-fetoprotein, lactic acid dehydrogenase and ACP were measured using the established methods developed by Forkasiewicz *et al.*,⁴⁷ Jasirwan *et al.*,⁴⁸ and Hong *et al.*⁴⁹

Statistic evaluation

To verify the statistical significance, a one-way Analysis of Variance (ANOVA) was utilized and then Tukey's *post hoc* test was also employed ($p < 0.05$). Mean \pm S.E. is used to express the values.

RESULTS

The percentage yield of the extract

All the phytoconstituents were extracted from HAESI extract (30:70) through the cold maceration technique. The phytoconstituents such as steroids, tannin, anthraquinone, polyphenol, glycosides, alkaloids, terpenoids, saponin, coumarins, triterpenoids, flavonoids were extracted from hydroalcoholic extract of *Syringodium isoetifolium* (Figure 2).

Phytochemical analysis

Chemical method

The phytochemical analysis of a hydroalcoholic extract of *Syringodium isoetifolium* was examined through chemical method and summarized in Table 1.

HRLCMS

There are 120 compounds were isolated in this method from the hydroalcoholic extract of *Syringodium isoetifolium* (Figure 3). Here, the phytoconstituents were identified through their molecular weight and typical mass fragmentation patterns. The prevailing compounds found in the mentioned extract are Phenophorbide A, Armillarin, Pyrophenophorbide A, Benzo (a) fluorene, Azelaic acid, 5-deoxy 5-methyl Sulfinyl adenosine, Corchorifatty acid F, Elaeocarpidine etc.,

Acute toxicity

The HAESI extracts at 500, 1000 and 2000 mg/kg body weight displayed no toxic effect on the experimental rats throughout the study. Neither lethality nor changes in behaviour were noticed. The acute toxicity results were furnished in Tables 2 and 3.

Table 1: Qualitative phytochemical analysis of leaves extracts of *Syringodium isoetifolium*.

Sl. No.	Phytochemicals	Hydro-alcoholic Extract (30:70%)
1	Tannin	++
2	Saponin	++
3	Flavonoids	++
4	Steroids	+
5	Terpenoids	+
6	Triterpenoids	+
7	Alkaloids	+
8	Anthroquinone	+
9	Polyphenol	++
10	Glycoside	+
11	Coumarins	+
12	Emodins	-
13	Anthocyanins	-
14	Carbohydrate	+
15	Carboxylic acid	+

("+" indicates presence of the compounds; "-" indicates absence of the compounds, "++" indicates the high concentration).

Effect of *Syringodium isoetifolium* on DEN induced hepatocellular carcinoma

Body and liver mass

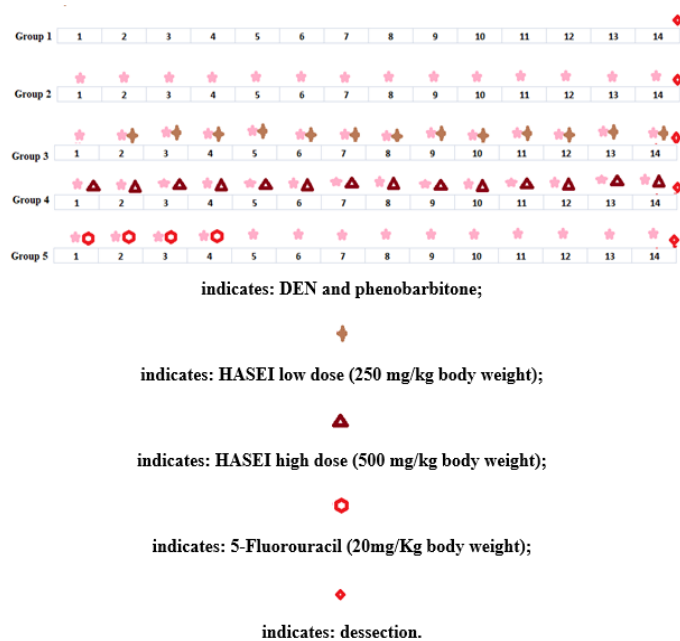
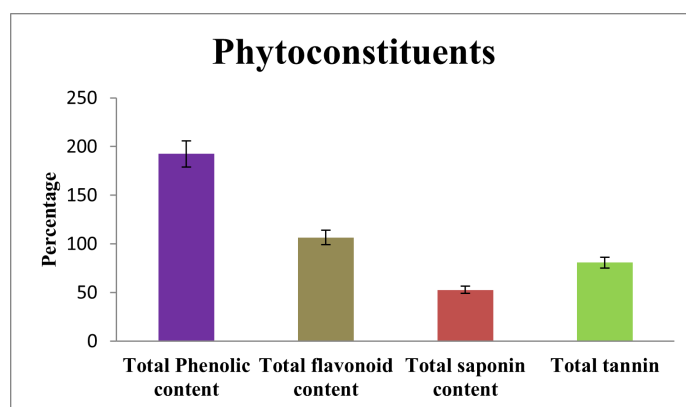
The body mass of rats was counted at the start and the end of the study. The body mass of rats improved considerably after treating with HAESI extracts. The liver mass was also weighed on the ninety-ninth day after the removal of the liver. The body and liver mass were shown in Table 4.

Estimation of haematological parameters

The effect of HAESI on the hematological parameters like red blood corpuscles, white blood corpuscles and Hemoglobin (Hb) was illustrated in Figure 4a. The DEN-treated group elevated levels of WBC (10.2 ± 0.318 , 10.8 ± 0.557) and decreased the levels of RBC (5.02 ± 0.123 , 5.28 ± 0.272) and Hemoglobin (12.4 ± 0.384 , 12.8 ± 0.176) in the blood. While the HAESI extracts at higher and lower doses administered to animals notably ($p < 0.001$) restored the entire hematological parameters. Hence the values of these parameters were near to Group I rats.

Effects on tumor number, volume and burden

It was evident from Figure 4b that rats from Group I exhibited no tumor. Whereas Group II animals displayed tumor number (6.67 ± 0.6), volume (4.57 ± 0.6) and burden (4.33 ± 0.8) due to the effect caused by DEN. When the HAESI (HD, LD) were given to groups III and IV, there is a considerable reduction in tumor

**Figure 1: Experimental protocol.****Figure 2: Phytoconstituents present in the hydroalcoholic extract of *Syringodium isoetifolium*.**

number (2.33 ± 0.33 , 2.67 ± 0.66), volume (2.77 ± 0.37 , 3 ± 0.05) and burden (1.67 ± 0.33 , 2.33 ± 0.66) amount respectively. Hence the number of tumors, volume and burden were controlled substantially.

Effect on total bilirubin, total protein, SGPT, SGOT and ALP

The Group II rats significantly raise the levels of total bilirubin (9.1 ± 0.17), total protein (13.5 ± 2.18), SGPT (89 ± 4.04), SGOT (99 ± 6.35), and ALP (308 ± 23.1) due to the effect caused by DEN. When the HAESI (HD, LD) were given to groups III and IV, there is a considerable restored amount of total Bilirubin (2.9 ± 0.289 , 2.2 ± 0.231), total protein (4.87 ± 1.17 , 5.9 ± 1.31), SGPT (40 ± 1.15 , 40 ± 2.31), SGOT (52 ± 3.46 , 80 ± 1.15) and ALP (203 ± 4.04 , 217 ± 16.7) respectively. Hence the amount of total Bilirubin, total protein, SGPT, SGOT and ALP was near to Group I animal (Figure 4c).

Table 2: Results of acute toxicity on Wistar albino rats.

Plant Extracts	Concentration (mg/kgbw)	Number of surviving albino Wistar rats									Percentage of Death
		1 st day			7 th day			14 th day			
		T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	
Hydroalcoholic extracts (HAESI).	500	03	03	03	03	03	03	03	03	03	0
	1000	03	03	03	03	03	03	03	03	03	0
	2000	03	03	03	03	03	03	03	03	03	0
	2500	03	03	03	03	03	03	03	03	03	0
	5000	03	03	03	03	03	03	03	03	03	0
Artificial Seawater (Control).	----	03	03	03	03	03	03	03	03	03	0

Table 3: Behavioral responses of female Wistar albino rats after administration of a single dose of hydroalcoholic extracts of *Syringodium isoetifolium*.

Observation	500 mg/kg	1000 mg/kg	2000 mg/kg
Body weight	Normal	Normal	Normal
Food intake	Normal	Normal	Normal
Water consumption	Normal	Normal	Normal
Temperature	Normal	Normal	Normal
Breathing	Normal	Normal	Normal
Irritability	No	No	No
Restlessness	No	No	No
Fearfulness	No	No	No
Skin colour	No change	No change	No change
Drowsiness	No	No	No
Death	Nil	Nil	Nil

Table 4: Effect of hydroalcoholic extracts of *Syringodium isoetifolium* on the body and liver weight of different rat's groups.

Group	Body Weight		Liver weight
	Initial Body Weight (gm)	Final Body Weight (gm)	
Group 1 (Normal control)	114±4.57 ^{**}	170±35.1 ^{**}	4.69±1.52
Group 1 (Diseased control)	103±2.56 ^{ns}	122±25.1 ^{ns}	6.84±1.42
Group 3 (DEN + HAESI L.D)	91±1.53 ^{**}	160±33.8 ^{**}	5.06±1.1
Group 4 (DEN + HAESI H.D)	116±1.71 ^{ns}	172±36.1 ^{**}	4.86±1.57
Group 5 (Den + STD drug)	128±4.7 ^{**}	175±58.3 ^{ns}	5.39±1.81

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant ** P< 0.05 calculated by comparing treated group with control group.

Effect on urea, creatinine and uric acid

The Group II rats significantly raise the levels of urea (93.7±2.63), creatinine (7.07±0.08), and uric acid (12.9±0.75) due to the effect caused by DEN. When the HAESI (HD, LD) were given to groups III and IV, there is a considerably restored amount of urea (50.5±1.21, 30.5±1.1), creatinine (2.75±0.491, 3±0.693) and uric acid (2.35±0.26, 4.55±0.202) respectively. Hence the amount of

urea, creatinine and uric acid was near to Group I animal (Figure 4d).

Effect on triglycerides, total cholesterol and HDL- cholesterol

The Group II rats significantly raise the levels of triglycerides (423±19.5), total cholesterol (320±23.1), and HDL cholesterol

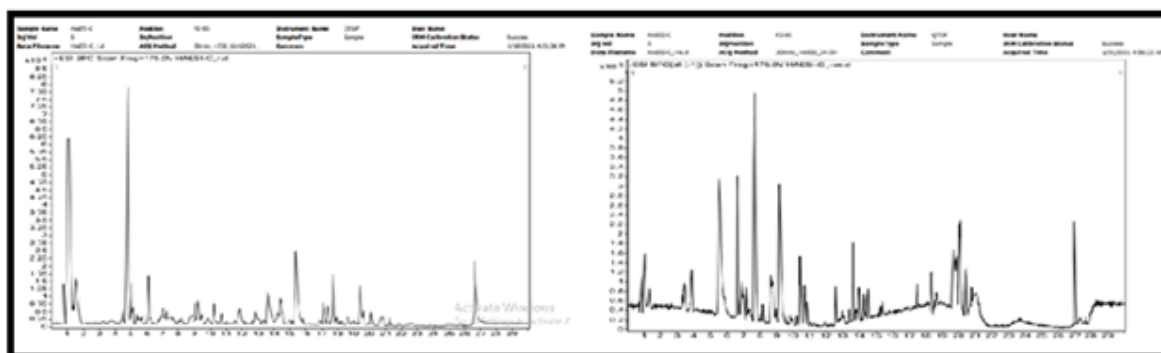


Figure 3: The chromatogram of hydroalcoholic extract of *Syringodium isoetifolium* in positive and negative run using HPLC.

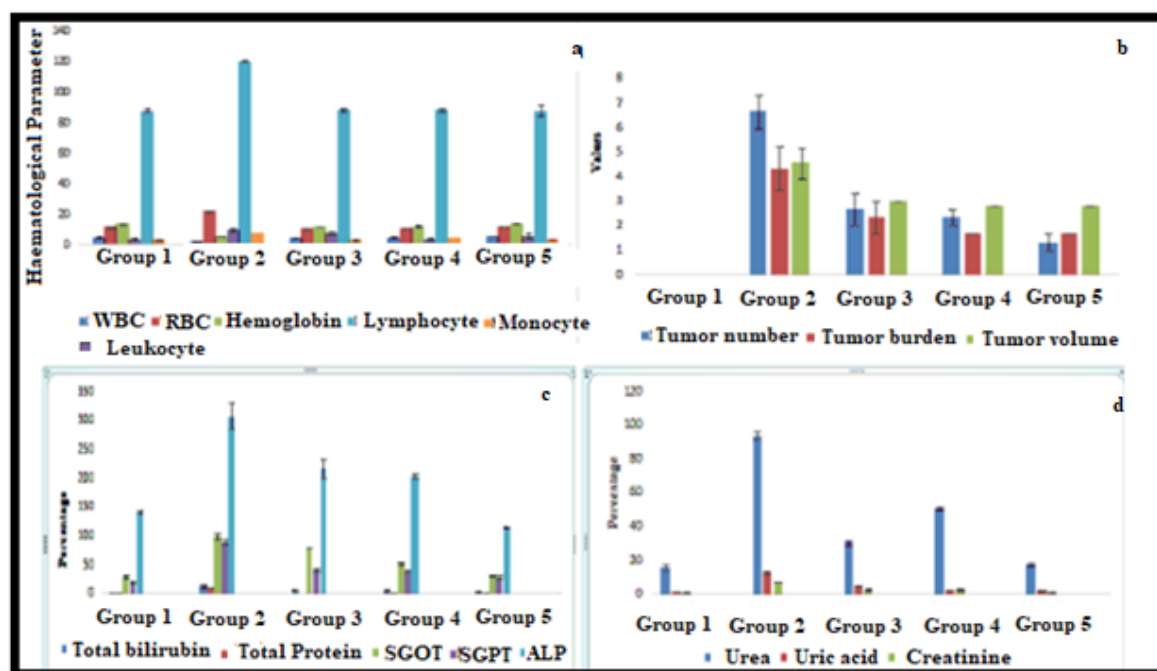


Figure 4: Effect of *Syringodium isoetifolium* on DEN induced hepatocellular carcinoma in Wistar albino rat (a-d).

(81.5 ± 1.82) due to the effect caused by DEN. When the HAESI (HD, LD) were given to groups III and IV, there is a considerably restored number of triglycerides (248 ± 5.31 , 163 ± 29.1), total cholesterol (168 ± 12.4 , 125 ± 8.66), and HDL cholesterol (39.6 ± 1.85 , 34.5 ± 1.21) respectively. Hence the number of triglycerides, total cholesterol and HDL-cholesterol was near to Group I animals (Figure 5a).

Effect on SOD, total protein, GPX, catalase and LPO

The Group II rats significantly raise the levels of SOD (0.741 ± 0.08), total protein (1.37 ± 0.03), GPX (0.267 ± 0.01), catalase (0.329 ± 0.07) and LPO (0.789 ± 0.02) due to the effect caused by DEN. When the HAESI (HD, LD) were given to groups III and IV, there is a considerable restored amount of SOD (0.354 ± 0.01 , 0.48 ± 0.02), total protein (0.874 ± 0.05 , 1.57 ± 0.19), GPX (0.123 ± 0.00 , 0.134 ± 0.01), catalase (0.179 ± 0.01 , 0.183 ± 0.00)

and LPO (0.511 ± 0.14 , 0.474 ± 0.04) respectively. Hence the amount of SOD, total protein, GPX, Catalase and LPO were near to Group I animals (Figure 5b).

Effect on Hexokinase (HK), Glucose-6-phosphatase (G6Pase), Glucokinase (GK), Fructose 1-6-di phosphatase (FBPase)

The Group II rats significantly raise the levels of HK (4.46 ± 0.06), G6Pase (78.5 ± 2.71), GK (0.161 ± 0.05) and FBPase (57.7 ± 0.49) due to the effect caused by DEN. When the HAESI (HD, LD) were given to groups III and IV, there is a considerably restored amount of HK (3.05 ± 0.31 , 4.77 ± 0.52), G6Pase (26.1 ± 2.17 , 27 ± 0.80), GK (0.139 ± 0.00 , 0.167 ± 0.00) and FBPase (29.9 ± 0.17 , 31.4 ± 1.62) respectively. Hence the amount of HK, G6PD, GK and Fru 1,6-P were near to Group I animals (Figure 5c).

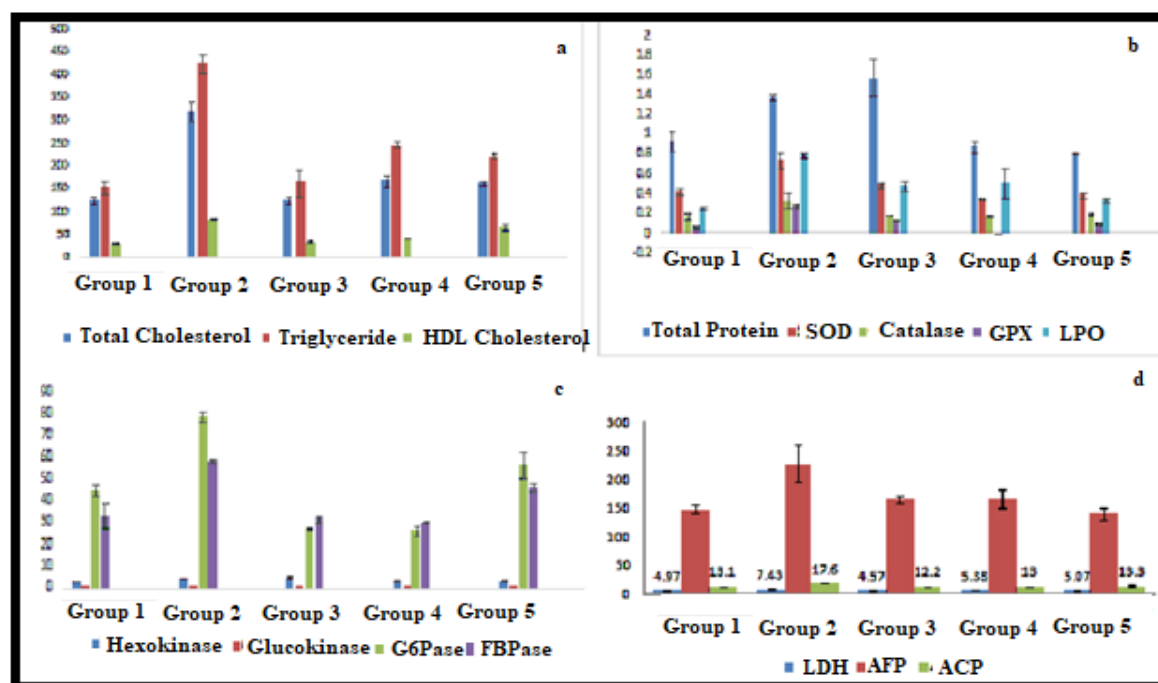


Figure 5: Effect of *Syringodium isoetifolium* on DEN induced hepatocellular carcinoma in Wistar albino rat (a-d).

Effect on Lactic Dehydrogenase (LDH), Alpha-Fetoprotein (AFP) and ACP

The Group II rats significantly raise the levels of LDH (7.43 ± 1.52), AFP (222 ± 32.2) and ACP (17.6 ± 1.24) due to the effect caused by DEN. When the HAESI (HD, LD) were given to groups III and IV, there is a considerably restored amount of LDH (5.33 ± 0.29 , 4.57 ± 0.57), AF (167 ± 16.80 , 166 ± 5.69) and ACP (13.0 ± 1.28 , 12.2 ± 0.87) respectively. Hence the amount of LDH, AF and ACP were near to Group I animals (Figure 5d).

Histopathological report

Transverse sections of the hepatic tissues Group II, III and IV were prepared and studied. Group II illustrated irregular cytoplasm, cell necrosis, multiloculated tumor giant cells, cytoplasmic vacuolation, hepatitis, central vein congestion, sinusoidal dilation and altered tubular architecture. HAESI-treated Groups (III and IV) showed improvement in liver tissue with improving cytoplasm, fewer necrosis cells, slightly altered hepatocytes and hepatocellular architecture. These changes were matched up to the reference group (5-Fluorouracil), which masks the outcome of diethyl nitrosamine (Figure 6).

DISCUSSION

Seagrass has tremendous biodiversity and source of natural phytoconstituents and exhibits different biological activities.^{50,51} It is the part of an aquatic ecosystem, found in seawater.⁵² Each of the secondary metabolites in seagrass exhibited diverse therapeutic activities after connecting with the particular receptor in our body.^{53,54} Former reports exhibited that *Syringodium isoetifolium*'s anti-cancer activity was due to the existence of

various phytochemicals such as alkaloids, tannin, saponin, flavonoids, anthocyanins, steroids, terpenoids, polyphenol, glycosides, emodin's, anthraquinone, coumarins, carbohydrates, carboxylic acid etc.,^{55,56} In this work, we prepared HAESI extracts and evaluated liver protective effects in experimental animals.

The diethylnitrosamine-induced tumor study is well recognized and often used in hepatocellular cancer study.⁵⁷⁻⁵⁹ Upon administration of DEN there is a considerable change in the serum constituents due to changes in the cell permeability thus fetching the leakage of cell constituents into the blood.⁶⁰ The increased level of these constituents proposed an increment in the structural changes in the physiology of the liver.⁶¹ Following the administration of HAESI extracts and reference drug, the body and liver mass, blood components, level of hormones and histological transforms were calculated in the tested rats at the conclusion of the study.⁶² The outcome exposed that HAESI extracts treated animals considerably change the body and liver mass based on the dose. The results were furnished in Table 4. The data were reliable to the former findings.^{63,64} In addition to that, the amount of tumor, burden and volume were evaluated to find the therapeutic effect of HAESI extracts against hepatocellular carcinoma.⁶⁵ Animals that were administered with the HAESI extracts significantly control the amount of tumor, burden and volume. The value is nearer to reference drug-treated animals. This result coincides with the earlier reports.⁶⁶⁻⁶⁸

Besides, the assessment of serum parameters is the key component to estimating the carcinoma in hepatic cancer. The experimental animals were administered with a higher and lower dose of HAESI extracts repaired the level of RBC, WBC and Hb

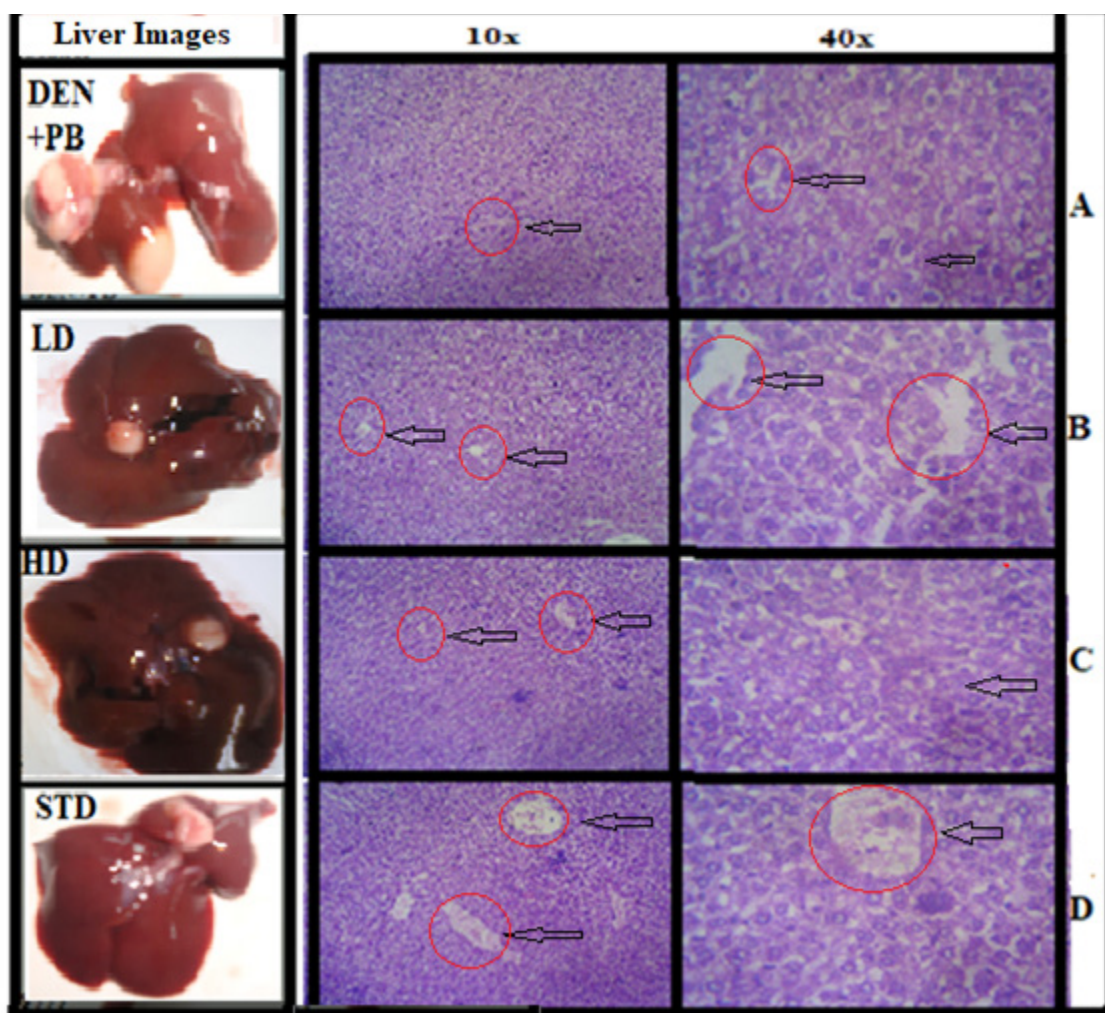


Figure 6: Effect of hydroalcoholic extracts of *Syringodium isoetifolium* on the rat livers at low and high doses A) Altered lobular architecture B) cytoplasmic vacuolation with interface hepatitis C) Dysplastic tumour cells D) Tumour cell with central vein congestion etc.

content in the tested animals. Whereas the Group II animals having reduced levels of red blood cells, Hb and elevated levels of white blood cells were identified. Earlier reports revealed comparable results with the hydroalcoholic extract of *Syringodium isoetifolium*.^{69,70}

Besides, HAESI extracts were used to calculate the blood-containing components and enzymes were calculated in the Group III and IV animals and documented the value of total bilirubin, total protein, SGOT, ALP, SGPT, urea, creatinine, uric acid, total cholesterol, HDL-cholesterol, triglycerides, LPO, SOD, total protein, GPX, catalase, glucokinase, glucose-6-phosphatase, hexokinase, fructose 1-6-di phosphatase. These amounts were smaller than the Group II animals. Both the blood parameters and levels of the hormone in the experimental rats were given in Figures 4, 5 (a-d). Comparable results were found in studies performed by other scientists who utilized *Enhalus acoroides* and *Cymodocea serrulata* and at different concentrations.^{71,72}

Assessment of tumor was further estimated by counting the number of biomarkers such as alpha-fetoprotein, lactic acid dehydrogenase and acid phosphatase. A lot of studies have shown that the mentioned biomarkers can encourage malignant growth during liver cancer.^{73,74} The HAESI extracts administered rats inhibit the amount of alpha-fetoprotein, lactic acid dehydrogenase and acid phosphatase. These values are lesser than the Group II animals. Hence, elevated levels of enzymes, blood parameters and biomarkers in the Group II animals due to improper cell permeability, metabolism and leakage of cell components into the blood when hepatocytes were damaged. It is a signal of acute necrosis in hepatic cells. The study recommended that the HAESI extract supplied a vast number of phytochemicals in the damaged liver and heal the tumor cells like a standard drug. Hence, it can be utilized as a new antitumor agent in liver cancer.

CONCLUSION

The work demonstrates that HAESI considerably manage tumor growth and normalizes the pathological parameters due to the existence of different phytochemicals in it. The cellular and metabolic changes were reversed in the HAESI treated groups as like a normal control group. The study strongly suggests that *Syringodium isoetifolium* is a promising anti-cancer agent with no plausible side effects.

ACKNOWLEDGEMENT

The Authors are thankful to the management of Vista, Pallavaram, GIET School of Pharmacy, Rajahmundry and KMCH college of Pharmacy, Coimbatore for offering the facilities required and also thankful to staffs of these institutes for providing their kind support to carry out this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ACP: Acid phosphatase; **AFP:** Alpha fetoprotein; **ALP:** Alkaline phosphatase; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **DEN:** N-Diethylnitrosamine; **Fbpase:** Fructose 1,6-bis phosphatase; **GK:** Glucokinase; **G6pase:** Glucose 6-phosphatase; **GPX:** Glutathione Peroxidase; **HAESI:** Hydroalcoholic extract of *Syringodium isoetifolium*; **HB:** Hemoglobin; **HD:** High dose; **HDL:** High-density lipoprotein; **HepG2:** Liver cell line; **HK:** Hexokinase; **LDH:** Lactic acid dehydrogenase; **LD:** Low dose; **LOP:** Lactoperoxidase; **PB:** phenobarbitone; **RBC:** Red blood cells; **SGPT:** Serum glutamic pyruvic transaminase; **SGOT:** Serum glutamic-oxaloacetic transaminase; **SOD:** Superoxide dismutase; **WHO:** World health organization; **WBC:** White blood cells.

SUMMARY

Cancer is defined as a new and diseased form of tissue growth. Cancerous cells may appear in one area and then spread via the lymph nodes. There was a 114.0% rise in liver cancer incident cases. Surgery, radiation, chemotherapy, targeted therapy, immunotherapy, bone marrow transplant, and hormone therapy are among the possible cancer treatments. One of the main problems with the development of traditional anti-cancer medications is the onset of multidrug resistance and relapse. Medicinal herbs and their phytoconstituents are being increasingly recognized as useful complementary treatments for cancer. The present study demonstrated the collection of *Syringodium isoetifolium* seagrass leaves, extraction with hydroalcoholic solvent and found out the *in vivo* anti-cancer activity against DEN-induced hepatocellular carcinoma in Wistar albino rats for

the first time. The findings suggest that the hydroalcoholic extract of *Syringodium isoetifolium* augments the antioxidant defence system and alters hematological parameters and liver glycolytic enzymes in DEN-induced cancers without producing side effects and resistance. It is a significant turning point in natural medicine used as an anti-cancer agent.

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Cite this article: Kavitha D, Padmini R, Dhanaraju MD, Gopi C, Thiyagarajan D, Veeramaneni A. *Syringodium isoetifolium* Fosters an Antioxidant Defense System, Modulates Glycolytic Enzymes and Protects Membrane Integrity in DEN-induced Hepatocellular Carcinoma in Albino Wistar Rats. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s690-s700.